

DESCRIPTION

PRODRUGS FOR USE AS OPHTHALMIC AGENTS

5 Cross-Reference to Related Applications

This application is a continuation-in-part application of U.S. Serial No. 10/405,413, filed April 1, 2003; which claims the benefit of U.S. provisional patent application Serial No. 60/369,589, filed April 1, 2002. This application also claims the benefit of U.S. provisional patent application Serial No. 60/432,354, filed December 9,
10 2002.

Field of the Invention

The present invention relates to prodrugs for use as ophthalmic agents, specifically for retinal protection. In particular, the present invention relates to the use of
15 steroidal quinols as prodrugs of phenolic A-ring steroid compounds to treat and/or prevent eye pathologies.

Background of Invention

A variety of tissues metabolize estrogen (as a representative phenolic A-ring
20 steroid) to various degrees. Of all of the tissues investigated, cornea appears to be the most active estrogen-metabolizing tissue (Starka, L and J Obenberger, "*In vitro* Estrone-Estradiol-17 β Interconversion in the Cornea, Lens, Iris and Retina of the Rabbit Eye," *Arch Klin Exp Ophthalmol*, 196:199-204 (1975)). Estrogens have demonstrated an important role in the health maintenance of all mucous membranes in the body, including
25 the maintenance of a healthy ocular surface. Additional studies have revealed that the biological activity of estrogen may be effective in the protection and treatment of the eye, including the lens and retina, against cataracts and the detrimental effects of glaucoma.

Unfortunately, many regions of the eye are relatively inaccessible to systemically administered estrogens. For example, orally administered estrogen passes through the
30 liver before reaching estrogen sensitive tissues. Because the liver contains enzymes that

can inactivate the estrogen, the estrogen that eventually reaches tissue targeted for treatment is virtually ineffective. Moreover, systemic administration of estrogen often produces undesirable side effects, *i.e.*, feminizing side effects in men.

As a result, topical drug delivery remains the preferred route of administration to the eye. There are a variety of factors that affect the absorption of drugs into the eye. These factors include: the instillation volume of the drug, the frequency of instilled drug administration, the structure and integrity of the cornea, the protein level in tears, the level of enzymes in tears, lacrimal drainage and tear turnover rate, as well the rate of adsorption and absorption of a drug by the conjunctiva, sclera, and eyelids.

Thus, the potential treatment of ocular disorders/conditions by estrogens or agents derived from estrogens is confounded by poor ocular bioavailability of pharmacologically active agents and by the likelihood of triggering systemic side effects associated with the administration of natural (endogenous) estrogens. The latter are due to absorption from the nasal cavity and the gastrointestinal (GI) tract after the topically administered estrogen hormone gains access to these pathways through its removal by the nasolacrimal apparatus of the eye. A potential way of reducing or even eliminating systemic side effects is to improve ocular targeting that would allow for the use of reduced doses of the biologically active agent in the ophthalmic drug formation.

Accordingly, the direct administration to an eye lens of estrogen having quinolines (*i.e.*, 6-hydroxyquinoline) and fused quinolines that act as steroid receptor modulators to prevent or treat cataract disorders has been disclosed. In addition, the administration of 17- β -estradiol to the surface of the eye to alleviate dry-eye syndrome or keratoconjunctivitis sicca has been disclosed. Glycosides of catechol estrogens have been formulated that demonstrate antioxidant activity to the same degree as to that of the parent catechol estrogens. Nonetheless, all of the previously disclosed compounds and methods for applying estrogens to the eye relate to compounds that lack efficient corneal penetration and/or are unapplicable to men because of their activity as a female hormone.

As noted above, the major barrier to ocular drug penetration is the cornea. The cornea is composed of three layers: a lipid-rich epithelium, a lipid-poor stroma, and a lipid-rich endothelium. Therefore, an agent must possess both lipophilic-hydrophilic

balance for adequate transcorneal penetration and, thus, ocular bioavailability (Akers HJ, "Ocular bioavailability of topically applied ophthalmic drugs," *Am Pharm*, NS23:33-36 (1983)). Thus, poor ocular bioavailability is an issue for estrogens and their synthetic analogs, because estrogens are highly lipid soluble molecules that are usually not amenable to adequate transcorneal penetration.

Prodrugs are inactive compounds that are converted *in vivo* into biologically active agents by enzymatic and/or chemical transformations. Prodrugs are advantageous because they can be designed to overcome problems associated with stability, toxicity, lack of specificity, or limited bioavailability, that may exist with the active form of a drug. Thus, there is a need to develop effective prodrugs of estrogen as a medical compound.

Estrogen quinols have been known for decades among organic chemists (Gold A.M., and Schwenk E., "Synthesis and reaction of steroidal quinols," *J Am Chem Soc*, 80:5683-5687 (1958)) though their metabolic formation has only been reported recently (Ohe T., *et al.*, "Novel metabolic pathway of estrone and 17 β -estradiol catalyzed by cytochrome P-450", *Drug Metab Dispos*, 28:11-112 (2000)). 10 β -hydroxy-1,4-estradiene-3,7-dione and 10 β ,17 β -dihydroxy-1,4-estradiene-3-one were detected from the respective estrogens during metabolic oxidation catalyzed by several cytochrome P-450 isoenzymes in rat liver microsomal systems. Contrary to well-known catechol metabolites of estrogens (Zhu, B.T. and Conney A.H., "Functional role of estrogen metabolism in target cells: review and perspective," *Carcinogenesis*, 19:1-27 (1998)), quinols do not possess an aromatic A-ring, making their biochemistry substantially different from that of catechols. Studies are currently underway to assess the nature of estrogen quinols.

Brief Summary

The subject invention provides materials and methods wherein unique and advantageous steroidal quinols are used for a broad range of therapeutic purposes, including the treatment or prevention of ophthalmic disorders and/or conditions by modulating or activating estrogen receptors. These disorders and/or conditions include,

but are not limited to, conjunctivitis, diabetic retinopathy, dry eye, glaucoma, and cataract.

A quinol (*i.e.*, the $10\alpha,\beta$ -hydroxyestra-1,4-diene-3-one structures) derived synthetically from phenolic A-ring steroids has been found to confer significant reduced lipid solubility compared to the parent phenolic A-ring steroid compounds to provide improved transcorneal penetration. Further, these quinols can be converted to phenolic A-ring steroid structures by endogeneous NAD(P)H as a reducing agent. In one embodiment, an oxidoreductase catalyst converts subject steroidal quinols to phenolic A-ring steroids that possess pharmacological activity in the eye. The present invention exploits the benefits of prodrugs (including but not exclusively based on the quinol structure as a novel pro-moiety) for phenolic A-ring steroid compounds to provide ocular bioavailability of the therapeutic agent in question. Prodrugs are, by definition, inactive compounds that are converted to the biologically active agents by chemical or enzymatic transformation *in vivo*.

The subject invention provides a mechanism by which quinol derived phenolic A-ring steroid compounds confer beneficial ophthalmic effects. The subject compounds possess a lipophilic-hydrophilic balance for transcorneal penetration and are readily reduced into parent phenolic A-ring steroid compounds to provide protection or treatment against various ocular symptoms and disorders. The compounds according to the subject invention appear to be highly advantageous as prodrugs to provide protection and/or treatment against ocular disorders. These prodrugs confer low lipid solubility and are readily converted by endogenous reducing agents into active phenolic A-ring steroid compounds. To the extent that these prodrugs have reduced feminizing effects and systemic toxicity, they would be expected to be quite advantageous for protecting or treating the eye against ocular disorders such as cataract or glaucoma.

In a specific embodiment, the subject invention provides steroidal quinol compounds that are, themselves, inactive. However, these quinol structures can act as prodrugs because they are converted to a therapeutically active phenolic A-ring steroid upon exposure to a reducing agent. Additionally, because an active phenolic A-ring steroid compound arises after conversion by a reducing agent, a smaller concentration of

the steroidal quinols is required as compared to direct administration of phenolic A-ring steroid, thus reducing the potential for systemic toxicity.

5 In one particular embodiment of the subject invention, isomers of 10-hydroxyestra-1,4-diene-3-one quinol structure (estrone-quinol) are converted to active, phenolic A-ring steroid compounds (*i.e.*, estrone) when exposed to a reducing agent. In related embodiments, quinols are derived from estrogen analogues, *i.e.*, 3,17-dihydroxyestra-1,3,5(10),9(11)-tetraene (ZYC1).

10 In another embodiment, steroidal quinols are provided as prodrugs that require at least one-step activation *in vivo* to yield pharmaceutically active estrogen compounds. In a related embodiment, quinols derived from estrogen prodrugs that require two-step activation can include a polar functional group to enhance hydrophilicity at the 17-OH group or may have the 10-OH group esterified to decrease lipophilicity through phosphate, or N,N,N-trialkylammonium esters.

15 In another embodiment, the 3,17-keto groups of quinols of the present invention can be functionalized as oxime and/or alkoximes. In doing so, preliminary compounds to the subject quinols are created (to form *i.e.* pro-prodrugs). Such functionalized quinols (*i.e.*, 3-keto functionalized as an oxime) can be used for a variety of therapeutic purposes, including use for ocular-specific delivery of phenolic A-ring steroids.

20 An object of the present invention is to provide compounds formulated for ophthalmic administration. For example, solutions or suspensions of these compounds may be formulated in the form of eye drops, or membranous ocular patch, which is applied directly to the surface of the eye.

25 It is another object of the subject invention to provide an ophthalmic agent with an increased therapeutic index associated with treatments using the subject compounds disclosed herein.

Brief Description of the Figures

30 **FIG. 1** illustrates the viability of retinal ganglial cells in the presence of glutamate, estrogen analog 3,17-dihydroxyestra-1,3,5(10),9(11)-tetraene (ZYC1), or combinations of glutamate and various concentrations of ZYC1.

FIG. 2 illustrates retinal ganglial cell viability when treated with glutamate in the presence or absence of ZYC1 or ZYC1 incubated in the presence of various concentrations of estrogen receptor antagonist, ICI164,384 (ICI).

FIG. 3 illustrates a quinol acetate in accordance with the subject invention.

5 **FIG. 4** illustrates an (alk)oxime of a quinol, in accordance with the subject invention.

FIG. 5 illustrates the viability of retinal ganglial cells in the presence of glutamate, phenolic A-ring steroid 2-(1-adamantyl)-3-hydroxyestra-1, 3, 5 (10)-trien-17-one (ZYC3), or combinations of glutamate and various concentrations of ZYC3.

10 **FIG. 6** illustrates LC/MS/MS analysis demonstrating the detection of 10 β -hydroxyestra-1,4-dien-3,17-dione (estrone-quinol, t_R =1.38) and a product formed from it (t_R =4.5 min.) after the incubation of estrone-quinol with NADPH.

FIG. 7 illustrates MS/MS analysis of the chromatographic peak at t_R =4.5 min., which is identical to that of estrone.

15 **FIG. 8** illustrates MS³ recording from the chromatographic peak, t_R =4.5 min., m/z 253 selected as precursor after MS/MS, which is identical to that of estrone.

Detailed Disclosure

20 The subject invention provides steroidal quinol compounds that produce phenolic A-ring steroids *in vivo*.. In one embodiment, these compounds provide improved physicochemical properties including, but not limited to, favorable ocular bioavailability and facile transcorneal penetration. In a preferred embodiment, estrogen derived quinol compounds demonstrate decreased lipophilicity as compared to lipophilic estrogens and estrogen analogues.

25 In another embodiment of this invention, these compounds treat and/or protect against various ocular diseases. Preferred compounds of the subject invention are effective in treating and/or preventing maladies associated with vision-threatening intraocular damage due to pathophysiological predispositions. Particularly preferred compounds are those which treat glaucoma and/or macular degeneration.

In a specific embodiment, the subject invention provides steroidal quinol compounds that are, themselves, inactive. However, these quinol structures can act as prodrugs because they are converted to a therapeutically active phenolic A-ring steroid upon exposure to a reducing agent. Additionally, because an active phenolic A-ring steroid compound arises after conversion by a reducing agent, a smaller concentration of the steroidal quinols is required due to their improved ocular bioavailability as compared to direct administration of estrogen, thus reducing the potential for systemic toxicity. In a related embodiment of the subject invention, steroidal quinols are provided as prodrugs that are converted into an active phenolic A-ring steroid via a one-step conversion by a reducing agent. Suitable reducing agents include endogenous NAD(P)H or oxidoreductases.

In one particular embodiment of the subject invention, a 10β -hydroxyestra-1,4-diene-3-one quinol structure (estrone-quinol) is converted to an active, phenolic A-ring estrogen compound (estrone) when exposed to a reducing agent. In related embodiments, quinols are derived from estrogen analogues, *i.e.*, 3,17-dihydroxyestra-1,3,5(10),9(11)-tetraene (ZYC1) or 2-(1-Adamantyl)estrone (ZYC3).

In another embodiment, steroidal quinols are provided as prodrugs that require two (or more than two) step activation *in vivo* to yield pharmaceutically active estrogen compounds. The liberation of a parent estrogen occurs through a two-step reaction: (1) enzymatic (phosphatase, esterase) cleavage of the ester group followed by (2) spontaneous and fast chemical conversion of a quinol by an endogenous reducing agent. In a related embodiment, these compounds according to the present invention can include a polar functional group to enhance hydrophilicity at the 17-OH group or may have the 10-OH group esterified to decrease lipophilicity through phosphate or N,N,N-trialkylammonium esters.

In another embodiment, the prodrugs according to the subject invention can be synthesized by attaching a polar functional group to enhance affinity to water and facilitate the transport of the prodrug of the subject invention through the lipid-poor middle stroma in the cornea. In a preferred embodiment, the 17-OH group of a quinol according to the subject invention is the primary site to which a polar functional group is

added. In another preferred embodiment, the 10β -OH of a steroidal quinol (*i.e.*, 17-hydroxyestra-1,4-diene-17-one) is blocked by esterification to make the resultant prodrug more lipophilic than the phenolic A-ring steroid derived quinol.

It will be noted that the structure of some of the compounds of this invention includes asymmetric carbon atoms. It is to be understood accordingly that the isomers arising from such asymmetry (*i.e.*, all enantiomers and diastereomers) are included within the scope of this invention, unless indicated otherwise. Such isomers can be obtained in substantially pure form by conventional methods including, for example, by classical separation techniques and by stereochemically controlled synthesis.

Definitions

A number of terms are used herein to designate particular elements of the present invention. When so used, the following meanings are intended:

The term "estrogen," as used herein, refers to both naturally occurring and synthetic substances classed as estrogen on the basis of their therapeutic or biological action (see listing under 'Estrogens' in the 'Therapeutic Category and Biological Activity Index' of The Merck Index, 12th Edition, Merck Research Laboratories, NJ, 1996, page THER-22). According to this listing, estrogens may be steroids (*i.e.*, estradiol, ethinyl estradiol, colpormon, conjugated estrogenic hormones, equilenin, equilin, estriol, estrone, mestranol, moxestrol, mytatrienediol, quinestradiol and quineestrol) or non-steroids (*i.e.*, diethylstilbestrol, dienestrol, benzestrol, broparoestrol, chlorotrianisene, dimestrol, fosfestrol, hexestrol, methallenestril, methestrol). Additional substances known to be estrogenic, that is, they interact with cellular estrogen receptors and mimic the effects of estrogens, include estrogenic substances that have been shown to be tissue selective in their estrogenic effects. Diverse classes of molecules fall within this category, for example: quinolines and fused quinolines that act as steroid receptor modulators such as 3,9-dihydroxy-5H-benzofuro[3,2-c]quinoline-6-one and those disclosed in WO 96/19458; phytoestrogens which occur naturally in plants such as forage plants, soya beans, seeds, berries and nuts (Jordan *et al.*, "Structure-activity relationships of estrogen," *Env. Health Per.*, 61:97-110 (1985)), including isoflavones such as genistein and genistein glycosides,

equol, O-desmethyl-angolensin, biochanin A, daidzein and formononetin; flavones such as phloretin, 4'-6-dihydroxyflavone and tricin, and coumestans such as coumestrol, 4'-O-methyl coumestrol, medicagol and sativol, lignans such as matairesinol, enterodiol, enterolactone, guaiaretic acid, nordihydroguaiaretic acid and derivatives thereof, β -sitosterol; mycoestrogens such as zeranol, zearalenol and zearalenone; estrogen receptor agonist/antagonists, such as tamoxifen, hydroxytamoxifen, zindoxifene and its metabolites, nafoxidene and derivatives, clomiphene, centchroman, benzothiophenes and related compounds such as benzothiophene-derived LY139478 (Eli Lilly), raloxifene and droloxifene, which may mimic the action of estrogens in certain types of cells, while opposing it in others (Raisz, L.G., "Estrogen and bone: new pieces to the puzzle," *Nature. Med.*, 2(10):1077-8 (1996)); and many para-substituted phenols that contain a strategically located phenolic hydroxyl not impaired by an alkyl substitution (see Jordan *et al.*, "Structure-activity relationships of estrogen," *Env. Health Per.*, 61:97-110 (1985)), including octyl phenyl, nonyl phenol, butylated hydroxyanisole, bisphenol A and trihydroxy-8-prenylflavone. Note that estrogenic substances in this general category may also be referred to in the literature as 'estrogens' (see Jordan *et al.*, 1985, for example). As already described above (for 'estrogens' as defined in Merck), estrogenic substances may exert their estrogenic effect(s) directly or they may require metabolic conversion to an active form after administration. For example, metabolic activation of some phytoestrogens involves demethylation to phenols (Jordan *et al.*, "Structure-activity relationships of estrogen," *Env. Health Per.*, 61:97-110 (1985)).

The term "estrogen derived quinols," (*i.e.*, $10\alpha/\beta$ -hydroxyestra-1,4-diene-3-one structure) as used herein, refers to quinols and quinol derivatives related to estrogens, as described above, and *para*-substituted phenols obtained by oxidation of the phenolic ring, as described below.

The term "phenolic A-ring steroid" used herein refers to compounds containing a 3-hydroxy-1,3,5(10)-triene moiety as the six-membered A-ring of a steroid, steroid analogue or steroid mimic, including compounds that manifest affinity to estrogen receptors (*i.e.*, 3,17-dihydroxyestra-1,3,5(10),9(11)-tetraene) as well as compounds that

do not bind to such receptors (*i.e.*, 2-(1-adamantyl)-3-hydroxyestra-1, 3, 5 (10)-trien-17-one).

The term “steroidal quinol” used herein refers to a steroid containing a 10 α / β -hydroxy-1,4-diene-3-one moiety as the six-membered A-ring of a steroid, steroid analogue or steroid mimic.

The term “ophthalmic disorders,” and/or “ophthalmic conditions,” as used herein, refers to ophthalmic diseases, conditions, and/or disorders including, without limitation, those associated with the anterior chamber of the eye (*i.e.*, hyphema, synechia); the choroid (*i.e.*, choroidal detachment, choroidal melanoma, multifocal choroidopathy syndromes); the conjunctiva (*i.e.*, conjunctivitis, cicatricial pemphigoid, filtering Bleb complications, conjunctival melanoma, Pharyngoconjunctival Fever, pterygium, conjunctival squamous cell carcinoma); connective tissue disorders (*i.e.*, ankylosing spondylitis, pseudoxanthoma elasticum, corneal abrasion or edema, limbal dermoid, crystalline dystrophy keratits, keratoconjunctivitis, keratoconus, keratopathy, megalocornea, corneal ulcer); dermatologic disorders (*i.e.*, eczematitis enteropathica, atopic dermatitis, ocular rosacea, psoriasis, Stevens-Johnson syndrome); endocrine disorders (*i.e.*, pituitary apoplexy); extraocular disorders (*i.e.*, Abducens Nerve Palsy, Brown syndrome, Duane syndrome, esotropia, exotropia, oculomotor nerve palsy); genetic disorders (*i.e.*, albinism, Down syndrome, Peters Anomaly); the globe (*i.e.*, anophthalmos, endophthalmitis); hematologic and cardiovascular disorders (*i.e.*, Giant Cell Arteritis, hypertension, leukemias, Ocular Ischemic syndrome, sickle cell disease); infectious diseases (*i.e.*, actinomycosis, botulism, HIV, diphtheria, Escherichia coli, Tuberculosis, ocular manifestations of syphilis); intraocular pressure (*i.e.*, glaucoma, ocular hypotony, Posner-Schlossman syndrome), the iris and ciliary body (*i.e.*, aniridia, iris prolaps, juvenile xanthogranuloma, ciliary body melanoma, iris melanoma, uveitis); the lacrimal system (*i.e.*, alacrima, Dry Eye syndrome, lacrimal gland tumors); the lens (*i.e.*, cataract, ectopia lentis, intraocular lens decentration or dislocation); the lid (*i.e.*, blepharitis, dermatochalasis, distichiasis, ectropion, eyelid coloboma, Floppy Eye syndrome, trichiasis, xanthelasma); metabolic disorders (*i.e.*, gout, hyperlipoproteinemia, Oculocerebrorenal syndrome); neurologic disorders (*i.e.*, Bell Palsy, diplopia, multiple

sclerosis); general ophthalmologic (*i.e.*, red eye, cataracts, macular degeneration, red eye, macular degeneration); the optic nerve (*i.e.*, meningioma, optic neuritis, optic neuropathy, papilledema); the orbit (*i.e.*, orbital cellulitis, orbital dermoid, orbital tumors); phakomatoses (*i.e.*, ataxia-telangiectasia, neurofibromatosis-1); presbyopia; the pupil
5 (*i.e.*, anisocoria, Horner syndrome); refractive disorders (*i.e.*, astigmatism, hyperopia, myopia); the retina (*i.e.*, Coats disease, Eales disease, macular edema, retinitis, retinopathy); and the sclera (*i.e.*, episcleritis, scleritis).

The term "patient," as used herein, describes an organism, including mammals, to which treatment with the compositions according to the present invention is provided.
10 Mammalian species that benefit from the disclosed methods of treatment include, and are not limited to, apes, chimpanzees, orangutans, humans, monkeys; and domesticated animals (*i.e.*, pets) such as dogs, cats, mice, rats, guinea pigs, and hamsters.

The term "polar aprotic solvent" refers to polar organic solvents lacking an easily removed proton, including, but not limited to, ethyl acetate, dimethylformamide (DMF),
15 and acetonitrile.

The term "pharmaceutically acceptable salts," as used herein, refers to those carboxylate salts, esters, and prodrugs of the compound of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals with undue toxicity, irritation, allergic response, and
20 the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention.

Pharmaceutically acceptable salts are well known in the art and refer to the relatively non-toxic, inorganic and organic acid addition salts of the compound of the present invention. For example, S. M. Berge, *et al.* describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 66:1-19 (1977) which is incorporated herein
25 by reference. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or separately by reacting the free base function with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid,
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hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxyethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

The term "pharmaceutically acceptable prodrugs," as used herein, refers to those prodrugs of the compounds of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention.

The term "prodrug," as used herein, refers to a derivative of a biologically active compound (*i.e.*, the steroidal quinols according to the present invention) that lacks pharmaceutical activity, but is converted (*i.e.*, by NAD(P)H) to an active agent, which is a phenolic A-ring steroid such as estrogen hormone, estrogen analogue, substituted estrogen or estrogen-receptor agonist or antagonist) upon interaction with a biological or chemical system, for example catalyzed reduction by enzymes in the eye. A thorough discussion is provided in T. Higuchi and V. Stella, Pro-drugs as Novel Delivery Systems,

Vol. 14 of the A.C.S. Symposium Series, and in Edward B. Roche, ed., *Bioreversible Carriers in Drug Design*, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated herein by reference. A prodrug, according to the present invention, can be converted into an active compound with one or more steps.

5 The term "substituted" shall be deemed to include multiple degrees of substitution by a named substituent. Where multiple substituent moieties are disclosed, the substituted compound can be independently substituted by one or more of the disclosed or claimed substituent moieties, singly or severally.

10 Unless otherwise specified, as used herein, the term "alkyl" refers to a straight or branched or cyclic alkyl moiety. In one embodiment, the alkyl moiety is C₁₋₂₀ alkyl, which refers to an alkyl moiety having from one to twenty carbon atoms, including for example, methyl, ethyl, propyl, isopropyl, butyl, *tert*-butyl, pentyl, hexyl and octyl, cycloalkyl including for example cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl. The alkyl group specifically includes fluorinated alkyls such as CF₃ and other halogenated
15 alkyls such as CH₂CF₂, CF₂CF₃, the chloro analogs, and the like. The alkyl group can be optionally substituted with one or more moieties selected from the group consisting of aryl, heteroaryl, heterocyclic, carbocycle, alkoxy, heterocycloxy, heterocylalkoxy, aryloxy; arylalkoxy; heteroaryloxy; heteroarylalkoxy, carbohydrate, amino acid, amino
20 acid esters, amino acid amides, alditol, halo, haloalkyl, hydroxyl, carboxyl, acyl, acyloxy, amino, amido, alkylamino, dialkylamino, arylamino, nitro, cyano, thiol, imide, sulfonic acid, sulfate, sulfonyl, sulfanyl, sulfinyl, sulfamoyl, carboxylic ester, carboxylic acid, amide, phosphonyl, phosphinyl, phosphoryl, thioester, thioether, oxime, hydrazine, carbamate, phosphonic acid, phosphate, phosphonate, phosphinate, sulfonamido, carboxamido, hydroxamic acid, sulfonylimide, substituted or unsubstituted urea
25 connected through nitrogen; or any other desired functional group that does not inhibit the pharmacological activity of this compound, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, *et al.*, *Protective Groups in Organic Synthesis*, John Wiley and Sons, Second Edition, 1991, hereby incorporated by reference.

The term “alkenyl” refers to a straight or branched alkyl moiety having one or more carbon double bonds, of either *E* or *Z* stereochemistry where applicable. This term includes for example, vinyl, 1-propenyl, 1- and 2- butenyl, and 2- methyl-2-propenyl, as well as “cycloalkenyl” groups such as cyclopentenyl and cyclohexenyl.

5 The term “alkoxy,” as used herein, and unless otherwise specified, refers to a moiety of the structure -O-alkyl, wherein alkyl is as defined above. The alkyl group can be optionally substituted as described above. Alkoxy groups can include OCF₃, OCH₂CF₃, OCF₂CF₃, and the like.

10 The term alkynyl refers to a hydrocarbon with at least one triple bond, including for example, C₁ to C₁₀ groups including but not limited to ethynyl, 1-propynyl, 1- and 2-butenyl, 1- methyl-2-butenyl, and the like.

15 The term “aryl,” as used herein, and unless otherwise specified, refers to phenyl, biphenyl, or naphthyl, and preferably phenyl. The aryl group can be optionally substituted with one or more of the moieties selected from the group consisting of alkyl, heteroaryl, heterocyclic, carbocycle, alkoxy, aryloxy, aryloxy; arylalkoxy; heteroaryloxy; heteroarylalkoxy, carbohydrate, amino acid, amino acid esters, amino acid amides, alditol, halo, haloalkyl, hydroxyl, carboxyl, acyl, acyloxy, amino, amido, alkylamino, dialkylamino, arylamino, nitro, cyano, thiol, imide, sulfonic acid, sulfate, sulfonyl, sulfanyl, sulfinyl, sulfamoyl, carboxylic ester, carboxylic acid, amide, phosphonyl, phosphinyl, phosphoryl, thioester, thioether, oxime, hydrazine, carbamate, phosphonic acid, phosphate, phosphonate, phosphinate, sulfonamido, carboxamido, hydroxamic acid, sulfonylimide or any other desired functional group that does not inhibit the pharmacological activity of this compound, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in *Greene, et al.*, “Protective Groups in Organic Synthesis,” John Wiley and Sons, Second Edition, 1991. Alternatively, adjacent groups on the aryl ring may combine to form a 5 to 7 membered carbocyclic, aryl, heteroaryl or heterocyclic ring.

25 The term “aralkoxy” refers to an aryl group attached to an alkyl group that is attached to the molecule through an oxygen atom. The aryl and alkyl groups can be optionally substituted as described above.

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The term "aralkyl," as used herein, and unless otherwise specified, refers to an aryl group as defined above linked to the molecule through an alkyl group as defined above. The aryl and alkyl portions can be optionally substituted as described above.

The term "aryloxy," as used herein, refers to an aryl group bound to the molecule through an oxygen atom. The aryl group can be optionally substituted as set out above for aryl groups. The terms "heteroaryl" and "heteroaromatic," as used herein, refer to monocyclic or bicyclic aromatic ring systems of five to ten atoms of which at least one atom is selected from O, N, and S, in which a carbon or nitrogen atom is the point of attachment, and in which one additional carbon atom is optionally replaced with a heteroatom selected from O or S, and in which from 1 to 3 additional carbon atoms are replaced by nitrogen heteroatoms.

Heteroaryl thus includes aromatic and partially aromatic groups that contain one or more heteroatoms. Examples of this type include but are not limited to are furan, benzofuran, thiophene, benzothiophene, pyrrole, pyrazole, imidazole, oxazole, benzoxazole, thiazole, benzthiazole, isothiazole, thiadiazole, triazole, benzotriazole, furazan, benzofurazan, thiafurazan, benzothiafurazan, tetrazole, oxadiazole, triazine, pyridine, pyridazine, pyrimidine, pyrazine, triazine, indolizine, indole, isoindole, purine, quinoline, benzimidazole, pteridine, isoquinoline, cinnoline, quinazoline, and quinoxaline.

The term "heteroaralkyl," as used herein, and unless otherwise specified, refers to a heteroaryl group as defined above linked to the molecule through an alkyl group as defined above.

The term "heterocyclealkyl," as used herein, refers to a heterocyclic group bound to the molecule through an alkyl group. The heterocyclic group and the alkyl group can be optionally substituted as described above. The term "heterocycloalkyl" can also refer to a saturated heterocyclic moiety having from two to six carbon atoms and one or more heteroatom from the group N, O, and S (or oxidized versions thereof) which may be optionally benzofused at any available position. This includes, for example, azetidiny, pyrrolidinyl, tetrahydrofuranyl, piperidinyl, benzodioxolyl and the like. The term "heterocycloalkyl" also refers to an alicyclic moiety having from three to six carbon

atoms and one or more heteroatoms from the group N, O, and S and having in addition one double bond. Such moieties may also be referred to as "heterocycloalkenyl" and includes, for example, dihydropyranyl, and the like.

The term "heterocyclic" refers to a nonaromatic cyclic group that may be partially (contains at least one double bond) or fully saturated and wherein there is at least one heteroatom, such as oxygen, sulfur, nitrogen, or phosphorus in the ring. The term heteroaryl or heteroaromatic, as used herein, refers to an aromatic that includes at least one sulfur, oxygen, nitrogen or phosphorus in the aromatic ring. Nonlimiting examples of heterocyclics and heteroaromatics are pyrrolidinyl, tetrahydrofuryl, piperazinyl, piperidinyl, morpholino, thiomorpholino, tetrahydropyranyl, imidazolyl, pyrrolinyl, pyrazolinyl, indolinyl, dioxolanyl, 1,4-dioxanyl, aziridinyl, furyl, furanyl, pyridyl, pyrimidinyl, benzoxazolyl, 1,2,4-oxadiazolyl, 1,3,4-oxadiazolyl, 1,3,4-thiadiazole, indazolyl, 1,3,5-triazinyl, thienyl, isothiazolyl, imidazolyl, tetrazolyl, pyrazinyl, benzofuranyl, quinolyl, isoquinolyl, benzothienyl, isobenzofuryl, pyrazolyl, indolyl, isoindolyl, benzimidazolyl, purinyl, carbazolyl, oxazolyl, thiazolyl, benzothiazolyl, isothiazolyl, 1,2,4-thiadiazolyl, isooxazolyl, pyrrolyl, quinazolinyl, cinnolinyl, phthalazinyl, xanthinyl, hypoxanthinyl, pyrazole, imidazole, 1,2,3-triazole, 1,2,4-triazole, 1,2,3-oxadiazole, thiazine, pyridazine, or pteridinyl wherein a heteroaryl or heterocyclic group can be optionally substituted with one or more substituent selected from the same substituents as set out above for aryl groups. Functional oxygen and nitrogen groups on the heteroaryl group can be protected as necessary or desired. Suitable protecting groups can include trimethylsilyl, dimethylhexylsilyl, *t*-butyldimethylsilyl, and *t*-butyldiphenylsilyl, trityl or substituted trityl, alkyl groups, acyl groups such as acetyl and propionyl, methanesulfonyl, and *p*-toluenesulfonyl.

The term "heteroaryloxy," as used herein, refers to a heteroaryl group bound to the molecule through an oxygen atom. The heteroaryl group can be optionally substituted as set out above for aryl groups.

The term "heterocyclealkoxy" refers to a heterocyclic group attached to an aryl group attached to an alkyl-O- group. The heterocyclic, aryl and alkyl groups can be optionally substituted as described above.

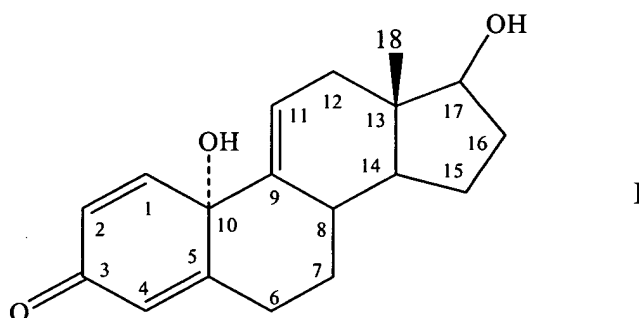
The term "electrolyte," as used herein, refers to salts generally and specifically to ions. An electrolyte refers to an ion that is electrically-charged, either negative or positive. Common electrolytes include chloride (Cl^-), bromide (Br^-), bicarbonate (HCO_3^-), sulfate (SO_4^{2-}), sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}).

Abbreviations

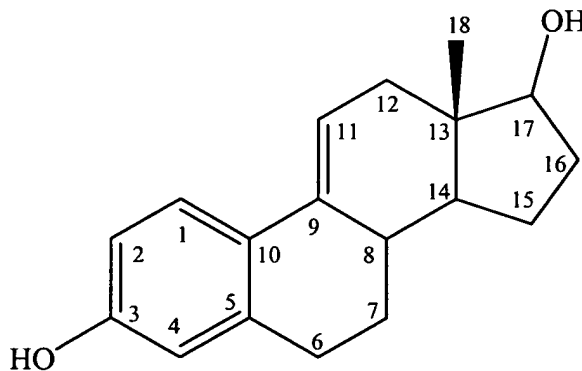
Abbreviations used in the examples are: DCC for 1,3-dicyclohexylcarbodiimide; DMAP for 4-dimethylamino-pyridine; LC/MS for liquid chromatography-mass spectrometer; *m*-CPBA for meta-chloroperoxybenzoic acid; and PhMe/EtOAC for toluene/ethyl acetate.

Steroidal Quinols

In one embodiment, a quinol of Formula I is provided as follows

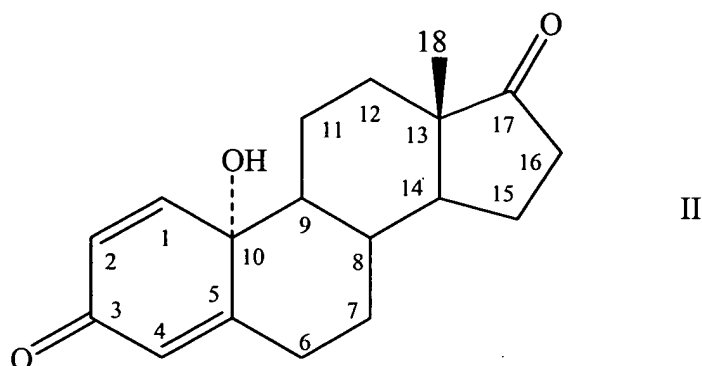


or a pharmaceutically acceptable salt or prodrug thereof; wherein the quinol of Formula I is derived from the following estrogen analogue (ZYC1)



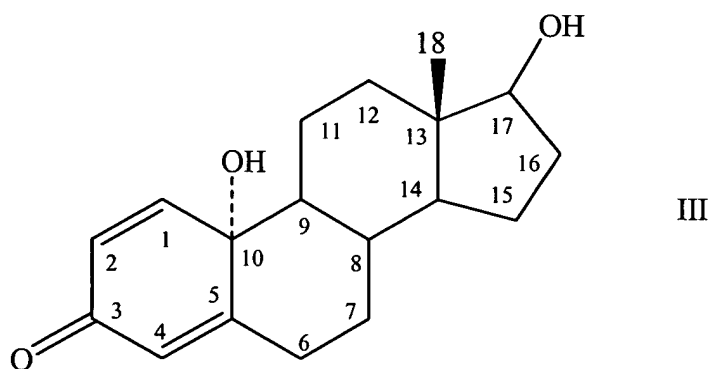
ZYC1 is an analogue of estrogen and has been demonstrated to have estrogen-like activity. The physicochemical properties of ZYC1 inhibit facile transcorneal penetration upon topical administration (*i.e.*, eye-drops). In accordance with the present invention, ZYC1 is oxidized to produce an steroidal quinol, 10,17-dihydroxyestra-1,4,9(11)-triene-3-one (“ZYC1-quinol”). The ZYC1-quinol, as discussed in more detail below, has demonstrated improved physicochemical properties, including decreased lipophilicity, to facilitate transcorneal penetration.

In another embodiment, a quinol of Formula II is provided as follows:



10 or a pharmaceutically acceptable salt or prodrug thereof, wherein the quinol of Formula II is derived from 3-hydroxyestra-1,3,5(10)-triene-17-one (estrone).

In yet another embodiment, a quinol of Formula III is provided as follows:



15 or a pharmaceutically acceptable or prodrug thereof, wherein the quinol of Formula III is derived from 3,17-dihydroxyestra-1,3,5(10)-triene (estradiol).

The compounds of Formulas I-III can also be functionalized at the 3- or 17-keto group as an oxime or alkoxime. Such compounds are useful as preliminary compounds to the quinol, for use as pro-prodrug compounds. These compounds would be useful for a variety of therapeutic purposes including, for example, use as a β -blocker.

5 The compounds and processes of the invention will be better understood in connection with the Examples, which are intended as an illustration of and not a limitation upon the scope of the invention.

Example 1 — Physicochemical Properties of ZYC1

10 Human retinal ganglial cells (RGC) were incubated with glutamate (5mM), the estrogen analogue 3,17-dihydroxyestra-1,3,5(10),9(11)-tetraene (ZYC1) or combination of glutamate and various concentrations of ZYC1. As illustrated in Figure 1, glutamate killed about 70% of RGC while the compound of Formula ZYC1 alone had no effect on RGC viability. In the presence of all three concentrations of ZYC1, glutamate killed
15 significantly fewer cells.

RGC were treated with glutamate (5mM) in the presence or absence of ZYC1. As illustrated in Figure 2, this estrogen analogue, ZYC1, reduced the number of RGC killed by glutamate. Where ZYC1 was incubated in the presence of various concentrations of estrogen receptor antagonist, IC1182,780 (ICI) (which at the lowest concentration used,
20 was more than 100-times its IC₅₀), little antagonism of ZYC1 protection of RGC was seen. This data suggests that ZYC1 protects RGC through a non-estrogen receptor mediated mechanism. However, the physicochemical properties of ZYC1 permit negligible transcorneal penetration upon topical administration.

Example 2 — Improved Physicochemical Properties of Steroidal Quinols

25 To test the hypothesis that directed modification of an estrogen improves physicochemical properties of transcorneal penetration, estrone was used as a lead compound. The following Table I indicates a very significant drop in lipophilicity of Formula I, Formula II, and Formula III, compared to the parent phenolic A-ring steroids,
30 ZYC1, estrone, and estradiol. The log of the *n*-octanol/water partitioning coefficient (log

P or log $D_{7,4}$) is the measure of attraction to lipid phase versus an aqueous phase. Log P is a crucial factor governing passive membrane partitioning, influencing permeability opposite to its effect on solubility (*i.e.*, increasing log P enhances permeability while reducing water solubility). Thus, the results of Table I demonstrate that the lipophilic-hydrophilic balance of Formula I, Formula II, and Formula III are closer to the ideal value for facile transcorneal penetration and favorable bioavailability than the parent phenolic A-ring steroids, ZYC1, ZYC3, estrone, and estradiol. It has been demonstrated that the ideal log P value for the brain is approximately 2. Though an ideal log P value for the cornea has not yet been determined, a log P value of two should be a reasonable value for the cornea.

TABLE I

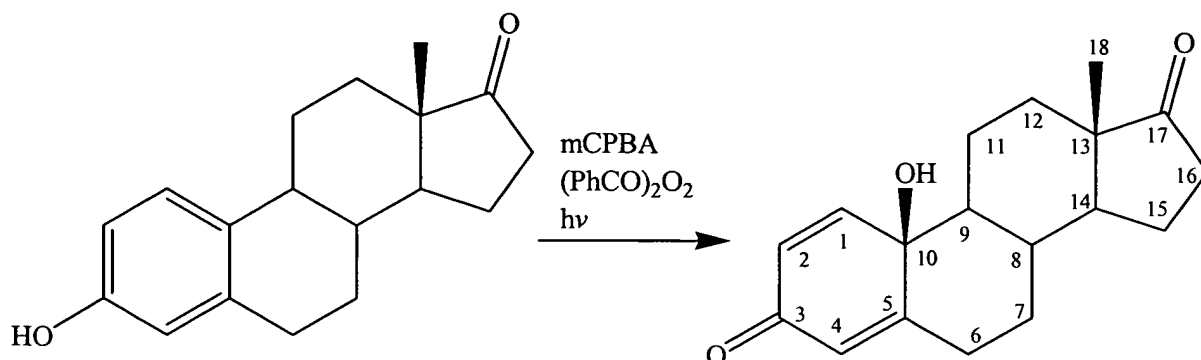
| COMPOUND | Log P | P |
|---|-------|-------------------|
| 3-hydroxyestra-1,3,5(10)-triene-17-one (estrone) | 4.54 | 64,670 |
| 3,17 β -dihydroxyestra-1,3,5(10)-triene (estradiol) | 4.01 | 10,230 |
| 3,17-dihydroxyestra-1,3,5(10),9(11)-tetraene (ZYC1) | 3.57 | 3,715 |
| 2-(1-adamantyl)-3-hydroxyestra-1,3,5(10)-triene-17-one [2-(1-adamantyl)estrone] (ZYC3) | 6.83 | $6.76 \cdot 10^6$ |
| 10 β -hydroxyestra-1,4-diene-3,17-dione ("estrone quinol") | 2.20 | 158 |
| 10 β ,17 β -dihydroxyestra-1,4-diene-3-one ("estradiol quinol") | 1.67 | 47 |
| 10 β , 17-dihydroxyestra-1,4,9(11)-triene-3-one ("Formula * quinol") | 1.30 | 20 |
| 2-(1-adamantyl)-10 β -hydroxyestra-1,4-diene-3,17-dione ["2-(1-adamantyl)estrone quinol"] (| 4.30 | 19,953 |

The log P values pertain to *n*-octanol/water partitioning were predicted by the method incorporated into CAChe WorkSystem Pro 5.0 (Fujitsu America, Inc., Beaverton, OR).

Example 3 — General Methods for Preparing a Steroidal Quinol

By way of example, Formula II (estrone quinol; 10 β -hydroxyestra-1,4-diene-3,17-dione) was prepared by the following Scheme I:

Scheme I



As understood by the skilled artisan, steroidal quinols according to the present invention may be synthesized using a “one-pot” phenol to quinol transformation. The synthesis method utilizes *m*-CPBA as an oxidant, dibenzoyl peroxide [(PheCO)₂O₂] as a radical initiator and visible-light irradiation that, in refluxing aprotic solvent, produces excellent yields of the quinols of the present invention.

By way of example, Solaja *et al.*, *Tetrahedron Letters*, 37:21, 3765-3768 (1996) discloses a “one-pot” method for synthesizing estrone-quinol. Oxidation of estrone to synthesize 10 β -hydroxyestra-1,4-diene-3,17-dione is performed by heating a stirred solution of estrone (10.00 g, 37.0 mmol), *m*-CPBA (22.53 g, 111.0 mmol; 85% Jansen Chimica), and (PheCO)₂O₂ (900 mg, 3.70 mmol) in 2L mixture of CCl₄/Me₂CO (4 / 1) to reflux for 3 hours while irradiated with a 60 Watt tungsten lamp. Upon evaporation of the solvent, extraction is performed with CHCl₃ (3 x 200 mL), washing with NaHCO₃ (2 x 100 mL) and H₂O (100 mL), and drying over anhydrous Na₂SO₄. The residue is then chromatographed on SiO₂ column. Elution may be performed with PhMe/EtOAc (1 / 1 and 7 / 3, respectively) and crystallization from benzene produces 5.19 g (49%) of estrone quinol as colorless needles.

Data regarding the resulting estrone quinols, as observed by Solaja *et al.* are as follows: mp = 219-221 °C (benzene); ¹H-NMR (250 MHz, DMSO-d₆): 7.13 (d, *J* = 10.4 Hz, H-C(1)), 6.07 (dd, *J* = 10.4, 2.4 Hz, H-C(2)), 5.92 (irreg. T, *J*_{4,2} = 2.4, *J*_{4,6 β} = 1.2 Hz, H-C(4)), 5.67 (s, H-o, exchangeable with D₂O), 2.67 (tdd, *J* = 15.2, 6.4, 1.2 Hz, H β -C(6)), 1.97-1.83 (m, H β -C(8) and H β -C(11)), 1.30-1.18 (m, H α -C(11)), 0.97 (s, H₃C-C(13)); ¹³C

NMR (62.9 MHz, DMSO- d_6): 220.33 (C(17)), 185.53 (C(3)), 165.09 (C(5)), 150.25 (C(1)), 128.30 (C(2)), 123.09 (C(4)), 70.10 (C(10)), 51.18 (C(9)), 50.10 (C(14)), 47.75 (C(13)), 35.62 (C(16)), 34.58 (C(8)), 32.19 (C(7)), 31.80 (C(6)), 31.03 (C(11)), 22.00 (C(12)), 21.90 (C(15)), 13.73 (C(18)); MS (EI, m/z): 286(M^+ , 84), 268(M^+ - H_2O , 39), 150(68), 145(100), 124(75), 107(50), 91(50), 79(54), and 55(60).

Alternatively, estrome quinols of the present invention can be prepared using 2-(1-adamantyl)-3-hydroxyestra-1, 3, 5 (10)-trien-17-one [2-(1-adamantyl)estrone], which can be made using methods previously described by Lunn, W.H. and E. Farkas, "The adamantly carbonium ion as a dehydrogenating agent, its reactions with estrone," *Tetrahedron*, 24:6773-6776 (1968). Estrone (270 mg, 1mmol) and 1-adamantanol (170 mg, 1mmol) were added to anhydrous *n*-pentane (6 mL) and the stirred mixture was cooled with an ice bath. Boron trifluoride etherate ($BF_3 \cdot EtOEt$, 0.4 mL) was added over a 10 minutes period. After an additional 15min, the ice bath was removed and stirring was continued for an additional 45 min at room temperature. During the 45 min, the solid present in the reaction mixture was dissolved and yellow oil formed. Crushed ice was then added while shaking and swirling the reaction flask and pink solid was formed. The filtered crude pink product was washed with water until the filtrate had a neutral pH and the solid was dried in a vacuum oven at 50 °C. The pink crude powder (0.4 g) was purified by flash chromatography (silica gel, eluted with 20% ethyl acetate in hexanes to yield the pure product; 0.31 g, 76.7%). The product was recrystallized from a mixture of chloroform and isopropyl alcohol and had: mp 322-324 °C, lit mp 295-296 °C; 1H NMR ($CDCl_3$, 300 MHz) δ 0.91 (s, 3H, C_{18} - CH_3), 2.8 (m, 2H, C_6 - CH_2), 4.71 (s, 1H, C_3 - OH), 6.42 (s, 1H, Aromatic H), 7.15 (s, 1H, Aromatic H).

2-(1-Adamantyl)estrone (also referred to herein as ZYC3) was oxidized with lead-acetate to the corresponding quinol acetate using the following procedures. To a solution of 3 g 2-(1-adamantyl)estrone in 50 ml of glacial acetic acid 11 g of lead(IV)-acetate was added. The solution was stirred at room temperature for 1 day. Then, the solution was concentrated *in vacuo* to an oil that was treated with 50 ml of water and 50 ml of chloroform. The organic layer was separated and washed with 10 % $NaHCO_3$ and water. After drying over Na_2SO_4 the chloroform was removed and the residue was purified by

column chromatography on silica gel using hexane ethyl acetate 4:1 (v/v) eluent. The pure quinol acetate (as illustrated in Figure 3), which is also a potential prodrug, has $R_f = 0.5$ on silicagel TLC with the same eluent. Typical resonances (ppm) in $^1\text{H-NMR}$ (CDCl_3) spectrum indicating the conversion to 2-substituted estrone quinol acetate were observed: 6.4 (s, 1H, H-1); 6.0 (s, 1H, H-4); 2.0 (s, 3H, 10- acetyl).

The quinol acetate (was then hydrolyzed to the quinol (where $R=\text{H}$; prepared as described above) in methanol using a slight excess of NaOMe in methanol (25 % w/v) overnight at room temperature. Then the solution was concentrated and glacial acetic acid was added to adjust the pH slightly acidic. Upon adding water the quinol precipitated out as a pale yellow solid that was again purified by column chromatography the same way as its acetate ($R_f = 0.53$). Typical resonances (ppm) in $^1\text{H-NMR}$ (CDCl_3) indicating the conversion to 2-substituted estrone quinol were observed: 6.6 (s, 1H, H-1); 5.9 (s, 1H, H-4). MS (EI): m/z 420 ($\text{M}^{+\bullet}$).

To prepare (alk)oxime estradiol quinols of the subject invention, such as those illustrated in Figure 4, 0.5 g of hydroxylamine hydrochloride is added to 0.5 g of estradiol quinol or alkoxyamine hydrochloride) in 5 ml of ethanol, 0.5 ml of pyridine was added and the solution was refluxed overnight. After cooling, the ethanol was removed and ice-cold water was added. The mixture was stirred until the oxime crystallized.

Example 4 — Physicochemical Properties of ZYC3

RGC were incubated with glutamate (5mM), with 2-(1-adamantyl)-3-hydroxyestra-1, 3, 5 (10)-trien-17-one (ZYC3), or with a combination of glutamate and various concentrations of ZYC3. As illustrated in Figure 5, glutamate killed about 70% of RGC while the compound of ZYC3 alone has no affect on RGC viability. In the presence of three different concentrations of ZYC3, glutamate killed significantly fewer cells (No statistically significant difference from RGC survival without exposure to glutamate).

Example 5 — Prodrug Activity

By way of example, conversion of Formula II by NAD(P)H as an endogenous reducing agent was tested. Estrone quinol (0.1 mM) and 1.0mM of NADPH or NADH in 0.1M sodium phosphate buffer (1 ml final volume, pH 7.5) was incubated at 37°C. At incremental time points, 100 μ l aliquots were removed into ice-cold centrifuge tubes, and 100 μ l of glacial acetic acid was added. After immediate extraction with ethyl acetate, the organic layer was evaporated under nitrogen stream. Reconstitution of the samples with the liquid chromatography mobile phase was followed by LC/MS analyses, the results of which are illustrated in Figures 6-8. For the control experiment, no reducing agent was used.

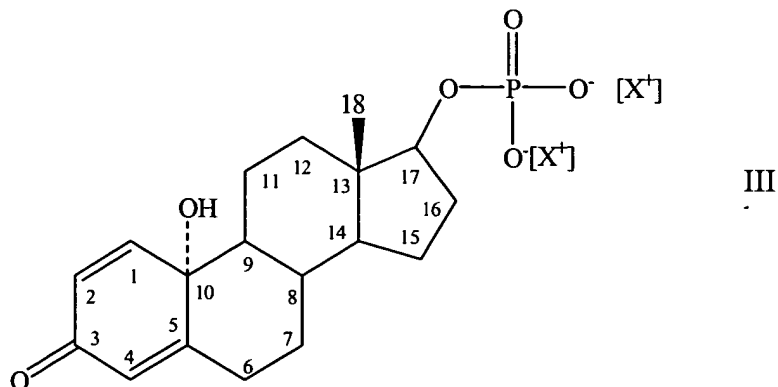
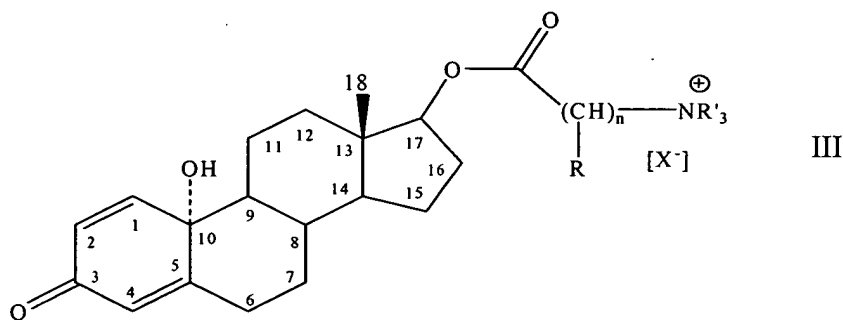
Liquid chromatography separation was done using a Supelco (Bellfonte, PA) 5 cm x 2.1 mm i.d. Discovery HS C-18 reversed-phase column with 0.25 ml/min water:methanol:2-propanol:acetic acid:dichloromethane (53:35:5:5:2, v/v) as a mobile phase. The sample residues were dissolved in 40 μ l of mobile phase, respectively, and 5 μ l of the solution was injected for analysis. Mass spectra were recorded on a quadrupole ion-trap instrument (LCQ[®], ThermoFinnigan, San Jose, CA) using positive-ion atmospheric-pressure chemical ionization (APCI) as the method of ionization. MS/MS and MS³ product-ion scans were obtained after collision-induced dissociation (CID) with helium as the target gas. Comparison with authentic reference compound (retention time, t_R , and mass spectra) was used for unambiguous identification of estrone. As an internal standard, 1,3,5(10)-estratrien-17 α -ethynyl-17 β -ol was added before each sample extraction. Estrone and estrone quinol levels were determined by LC/APCI-MS/MS and calibration with solutions of known concentrations of estrone (0.02 μ M to 11 μ M) and estrone quinol (0.2 μ M to 125 μ M) extracted for analyses. The chromatographic peak areas for estrone and estrone quinol were obtained from m/z 271 \rightarrow 253 and m/z 287 \rightarrow 269 MS/MS transitions, respectively. Formation of estrone was clearly detectable even after a short period of time, when the incubation was carried out in the presence of NADH and, especially, NADPH.

The rate of conversion at 37 °C and with a 10-fold excess of the ubiquitous reducing agent NADPH is $6.0 \times 10^{-7} \pm 4 \times 10^{-8} \text{ M} \cdot \text{min}^{-1}$, which indicates a rapid process

required for the proposed action of a quinol as a prodrug. Enzymes may also catalyze reductions in the eye. See Sichi H and D.W. Nebert, "In: Extrahepatic Metabolism of Drugs and Other Foreign Compounds (Gram TE, Ed.)," S.P. Medical and Scientific Books, New York, pp. 333-363 (1980), and Starka L and J. Obenberger. (In vitro estrone-estradiol-17 β interconversion in cornea, lens, iris and retina of rabbit eye," *Arch Klin Exp Ophthalmol*, 196:199-204 (1975).

Example 6 — General Methods for Preparing Prodrugs

In general, where a steroidal quinol according to the subject invention contains a hydroxyl group (*i.e.*, 17-OH group or 10 β -OH group), an "ester" moiety can replace the hydroxyl portion to form a non-acidic (neutral) ester compound. The addition of a polar functional group (*i.e.*, tertiary amide or phosphate ester) enhances the phenolic A-ring steroid-derived quinol's affinity to water and thus facilitates the transport of the quinol through the lipid-poor soma in the cornea. The following compounds of Formula III, Formula IIIa and Formula IIIb, illustrate polar functional groups attached at the 17-OH group.



wherein

each R and R' is independently hydrogen, alkyl, alkenyl, alkynyl, alkoxy, aryl, aralkoxy, aralkyl, aryloxy, hydroxyalkyl, alkoxyalkyl, heteroaralkyl, heterocyclealkyl, heteroaryloxy; and heterocyclearalkoxy;

5 X is an electrolyte; and

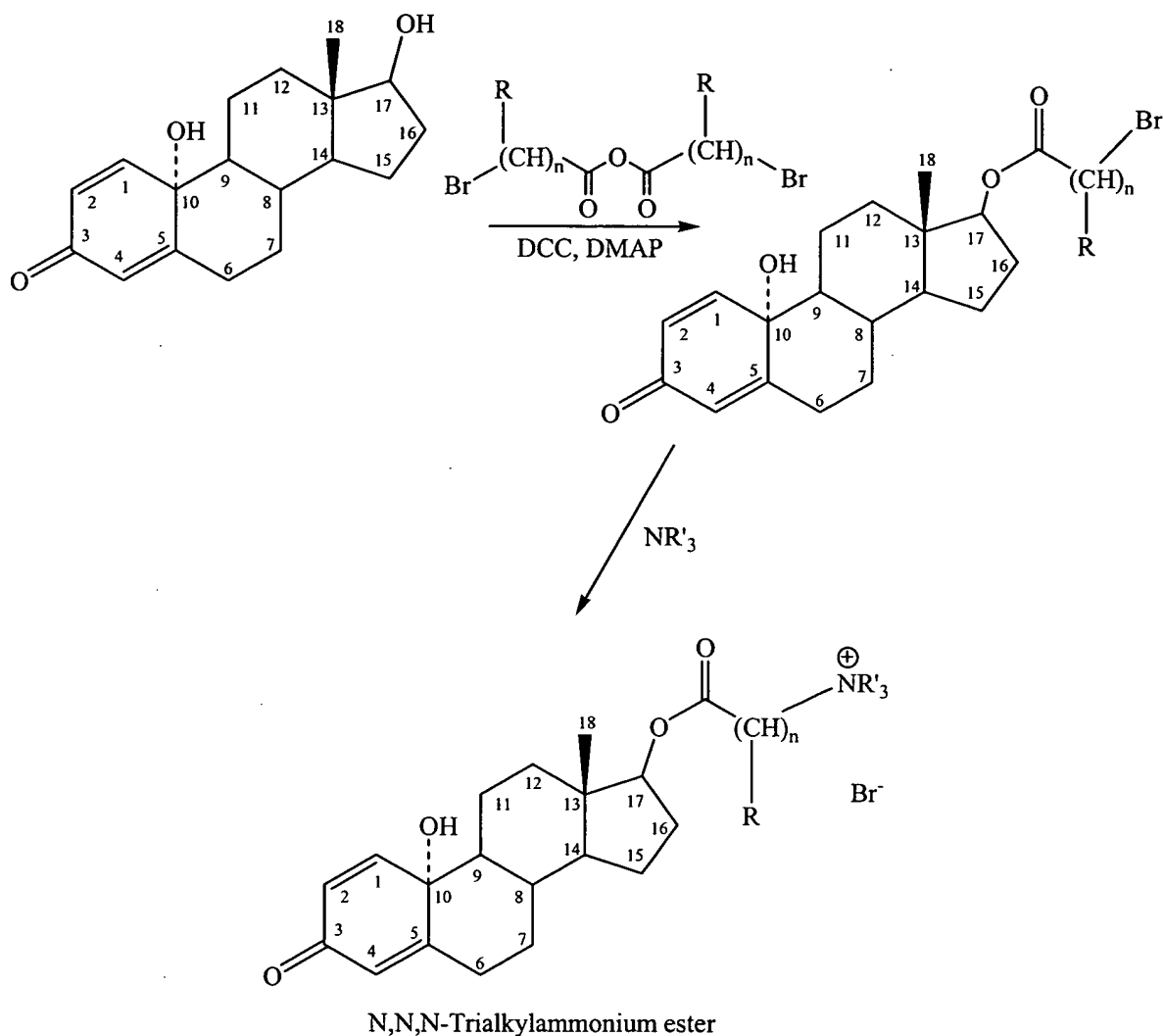
n is an integer from 1 to 20.

By way of example, a prodrug of Formula I (n=1, R=H) may be obtained by converting Formula III into an ester compound as illustrated in the following Scheme IIa.

10 To a solution of 10 β ,17 β -dihydroxyestra-1,4-diene-3-one (Formula I, estradiol quinol) in chloroform or ethyl acetate bromoacetic anhydride, DCC, and DMAP are added. The resulting mixture is stirred at 20-25 °C for 48 hours. The organic solution is extracted with water then dried over Na₂SO₄ and evaporated. The residue is purified by chromatography (silica gel: Aldrich, Merck grade 60, 230-400 mesh, 32 x 2 cm; elution with hexane containing gradually increasing concentrations of ethyl acetate from 0 to

15 6%). The purified residue in hexane is then placed in a closed system under argon, and trimethylamine (gas) was added at 20-25 °C then the precipitate was filtered, and rinsed with hexane. The resultant prodrug of estradiol quinol (10 β ,17 β -dihydroxyestra-1,4-diene-3-one-17-acetyl-trimethylammonium bromide) should have adequate solubility and sufficient stability to allow for formulation and storage. Further, the exemplary prodrug

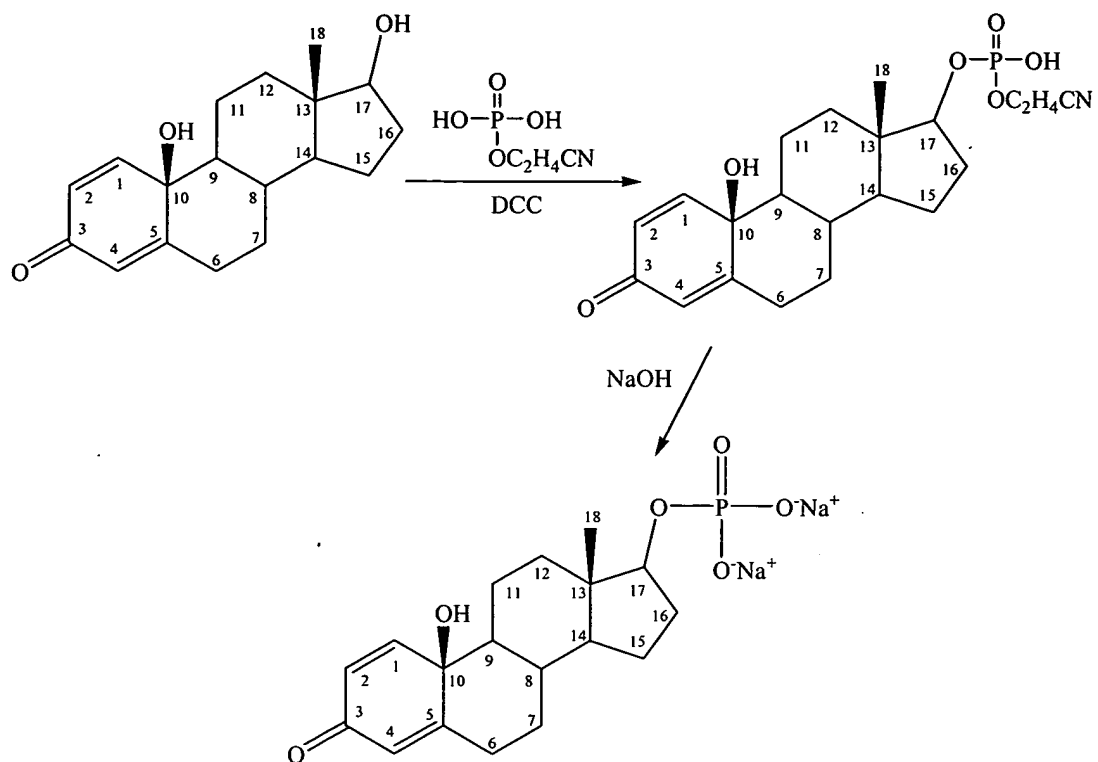
20 of estrone quinol is easily converted through an enzymatic or chemical process to the active compound, estrone, within the body, preferably the eye. In the following Scheme IIa, a prodrug of Formula I can be obtained

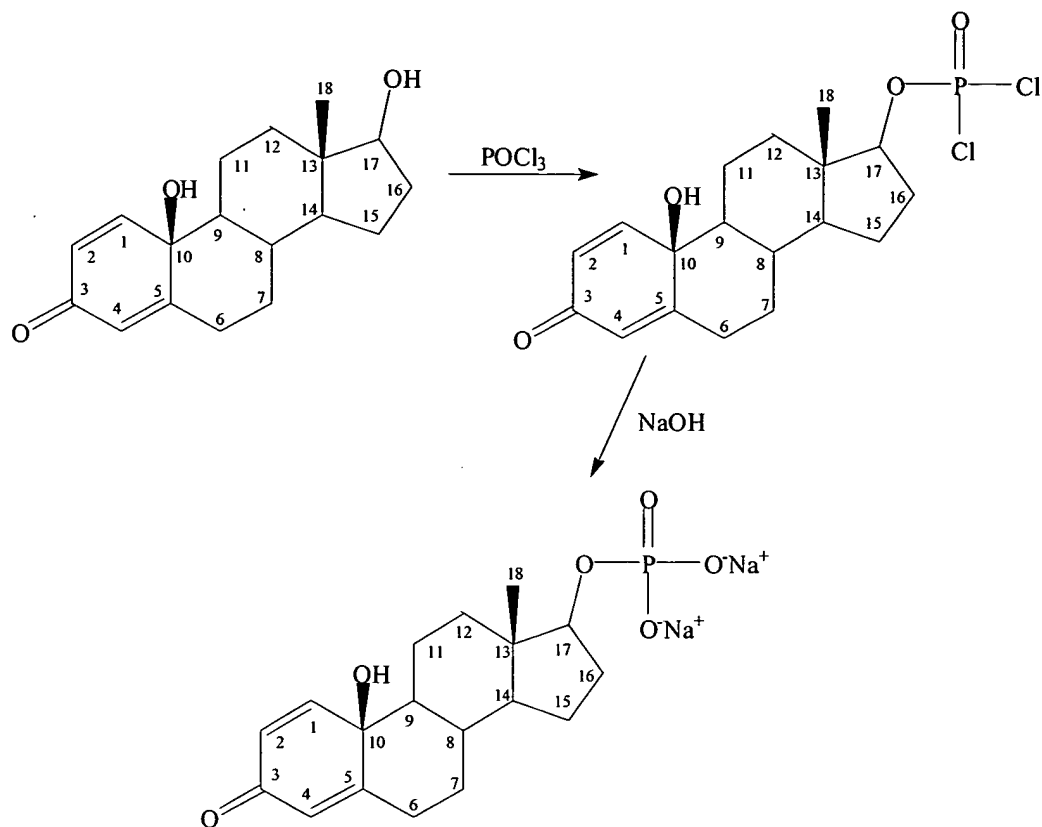
Scheme IIa

wherein R, R', X, and n are as defined above.

Phosphate esters can also be attached as a polar functional group to enhance water affinity of steroidal quinols. For example, phosphate ester prodrugs of estrogens according to the present invention can be prepared by an ester linkage to one of the hydroxyl groups of the head group of an steroidal quinol.

By way of example, the prodrug of estradiol, in accordance with the present invention, may be prepared using general methods as depicted in the following Schemes IIIa and IIIb.

Scheme IIIa

Scheme IIIb

5 All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

10 It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.